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MYCOLOGY. —*Three new species of* Conidiobolus *isolated from leaf mold.* CHARLES DRECHSLER, U. S. Department of Agriculture, Plant Industry Station, Beltsville, Md.

Recently I reported (Drechsler, 1952) that *Delacroixia coronata* (Cost.) Sacc. & Syd., a saprophytic entomophthoraceous fungus which earlier was encountered only rarely by mycologists and was generally presumed to be very meagerly distributed, is in fact virtually ubiquitous on leaf mold and other vegetable materials undergoing slow decay in moist contact with the ground. Separate cultures of the fungus, free of alien organisms, are with little effort obtainable in large numbers from isolation plate cultures prepared by fastening portions of decaying plant detritus with soft agar in a central area on the ceiling of each Petri dish. The soft agar employed not only serves as an adhesive matrix securely holding all particles of detritus in a canopylike layer about 10 mm above the layer of sterile agar on the floor of the Petri dish, but also supplies moisture to all detritus particles and thereby encourages prompt germination of any conidia or resting spores that may be present. Since in *D. coronata* either repetitional or mycelial development soon leads to formation and violent discharge of new conidia, macroscopically discernible mycelia of this fungus are commonly found growing in a maizemeal-agar plate within 48 hours after the canopy of leaf mold has been superposed. From the regularity with which *D. coronata* develops in canopied agar plates, even though only 0.2 to 0.3 gram of leaf mold is used in each Petri dish, it would seem beyond question that this fungus must exist in our middle and northern latitudes more abundantly than any of the numerous conspicuously insectivorous species through which the Entomophthoraceae have long been familiar.

The frequently early appearance of *Delacroixia coronata* in canopied plate cultures, together with its rapid growth, its prompt production of numerous conidia, and the forceful projection of these conidia over adjacent areas, makes more difficult the detection and isolation of less vigorous entomophthoraceous fungi likewise commonly present in leaf mold and other slowly decaying residues. Mainly for this reason few cultures referable to *Conidiobolus* were obtained from several dozen of the first canopied agar plates I prepared with leaf mold from different localities in Maryland and Virginia. The difficulties consequent to excessively close seeding of the conidia on the sterile substratum were later obviated with fair success by leaving agar plates exposed to conidial discharge for only a few hours, especially during the third and fourth days after the canopy had been prepared. Conveniently sparse seeding was obtained by removing the lid and its adhering canopy at successive intervals to a new bottom containing a newly poured plate of sterile maizemeal agar, each agar plate after exposure being immediately covered with a sterile lid. After 6, 8, or 10 hours, when some of the scattered conidia had grown out vegetatively, the resulting mycelia could be detected readily by examining the agar surface with the naked eye by reflected light. Through early removal of the young mycelia to sterile maizemeal agar slants plenteous collections of pure cultures were obtained; in these were included, besides *D. coronata* and some almost equally vigorous entomophthoraceous species, a number of related species less obtrusive because of their

slower growth and feebler conidial propulsion.

Most of the entomophthoraceous fungi thus isolated seem best assignable to the genus *Conidiobolus* erected by Brefeld (1884) primarily on his *C. utriculosus*, a robust species that made its appearance adventitiously in nutrient solution he had placed under fruiting bodies of *Hirneola* and *Exidia* for the purpose of germinating discharged basidiospores. From the scale of magnification indicated for the relevant figures, the disjunctive mycelial hyphae of this species seem to vary from 10 to 20μ in width. Its globose zygospores are stated to measure 60 to 100μ in diameter. Its conidia are described as being pear-shaped, with a length of 50μ and a width of 35μ. Although such large dimensions should help to invite notice, *C. utriculosus* has apparently not been recorded again at first hand since its description 68 years ago. Brefeld also reported as occurring on some "Tremellinen" a second species of *Conidiobolus* with conidia he stated to be scarcely one-third as large as those of *C. utriculosus*. The few illustrations he gave of these smaller spores show lengths varying from 20 to 23μ and widths varying from 14 to 15μ. Since in his material the smaller species always became overgrown at an early stage by the more vigorous *C. utriculosus* he was unable to cultivate it separately, and only rather provisionally named it *C. minor*. No additional first-hand report of this tentative species is known. In view of the circumstances under which it was observed its distinctness from *C. utriculosus* is open to serious doubt, for in all fairly robust species continued repetitional development leads to marked reduction in conidial size and indeed often brings about dimensional differences more pronounced than the differences noted by Brefeld. On the other hand, if the assemblage of entomophthoraceous fungi I have so far isolated from decaying plant detritus is at all representative, species with relatively small primary conidia are more numerous than species rivaling *C. utriculosus* in the size of their asexual spores.

Nevertheless, a saprophytic member of the Entomophthoraceae that appears even more robust than *Conidiobolus utriculosus* was obtained by Gilbert (1919) from fern prothallia grown in water cultures or on moist sphagnum. The large globose primary conidia of this fungus, which are described as measuring 48 to 60μ in diameter, would seem alien both to *C. utriculosus* and to *Delacroixia coronata*. The propulsion of these conidia often for a distance of 65 mm bespeaks a discharge mechanism several times more powerful than any mechanism operative in the different species of my collection. As Gilbert's account makes no mention of hirsute resting spores or of any production of small conidia on multiple short outgrowths extended from large conidia, the fungus may eventually find a place in Brefeld's genus. Apparently it has not been reported again during the 33 years since it was first made known and has not hitherto appeared among my cultures.

More recently Couch (1939) fully described under the binomial *Conidiobolus brefeldianus* a readily culturable entomophthoraceous fungus he obtained as a contaminant in an agar plate that had been exposed to spore discharge from a fruiting layer of *Septobasidium apiculatum* Couch on *Cornus amomum* Mill. From *Conidiobolus utriculosus*, with which it makes up the meager established membership of the genus, this fungus differs by its generally smaller dimensions—the width of its hyphae being given as varying from 5.4 to 8μ, the thickness of its spherical conidia as varying from 10 to 31μ, and the diameter of its zygospores as varying from 18 to 33μ. Canopied agar plate cultures prepared with plant detritus taken from localities near the District of Columbia during the winter of 1951–52 have not yielded *C. brefeldianus*, but the species has come forth abundantly in cultures prepared with small quantities of some dry plant detritus which W. F. Jeffers kindly collected early in July 1951 in woods near Tampa, Fla., and near Statesboro, Ga.

While canopied agar plate cultures are very serviceable in bringing to light a category of entomophthoraceous fungi that are not often encountered by chance, and in showing such fungi to be virtually ubiquitous on slowly decaying detritus, they are far

less helpful than might be desired in disclosing what particular constituents of detritus samples were used as sources of nourishment. Owing to the forceful discharge of conidia by the fungi in question, and to successive repetitional development of the discharged spores, it may be presumed that during prolonged periods of rainy weather numerous constituent particles near each particle used as a nutrient substratum will become bestrewn with conidia in greater or lesser abundance. Naturally when samples of detritus are gathered before drier conditions have supervened, and portions of them are fastened soon afterwards in a moist matrix to the ceiling of a Petri plate, some of the conidia adhering to merely contaminated particles may be expected to produce and shoot off new conidia that will be no less effective in establishing mycelia on the agar below than conidia shot off from the nutrient particles themselves. It is true that if canopied cultures are prepared with detritus that has previously been exposed for several months to gradual drying, all the earliest new conidia may be expected to have their origin from the germination of resting spores, which, except in *Delacroixia coronata*, are commonly formed on the assimilative hyphae, and therefore should be present mainly in nutrient particles. Such germination, however, entails some delay, so that when the earliest new conidia fall on the agar floor the whole canopy has become so badly overgrown with alien molds that the individual particles are obscured beyond recognition.

Although the species of Entomophthoraceae readily growing in pure culture on ordinary substrata are often termed "saprophytic" they do not, as a rule, thrive well in the presence of putrefactive organisms. Even rather slight bacterial contamination often halts their vegetative development completely, and subsequently brings about degeneration of their mycelial hyphae and asexual reproductive apparatus throughout the affected area. In agar plate cultures exposed to promiscuous contamination they are often rather strongly repressed by filamentous fungi little noted for antagonistic behavior. Against generally antagonistic molds, as, for example, species of *Penicillium*, *Aspergillus*, and *Trichoderma*, they show, on the whole, very little endurance. Rather commonly when their conidia fall near such molds neither vegetative nor repetitional germination ensues, but the spores turn dark and degenerate internally.

Despite the wide assortment of filamentous fungi with which they were often intermixed in older plate cultures, none of the species in my collection have been seen attacking other molds. In view of Brefeld's statement that *Conidiobolus utriculosus* under natural conditions subsisted parasitically on fruiting bodies of "Tremellinen" occasion was taken whenever possible to observe the behavior of *Conidiobolus* mycelia when they encountered mycelia of basidiomycetes. Suitable opportunity for such observation was offered frequently in agar plate cultures that had been canopied with fine-textured debris found lodged basally between the crowded culms in old tussocks of some grasses, for in addition to conidia of *Conidiobolus* this kind of litter brought forth basidiospores that likewise were discharged early and gave rise to numerous clamp-bearing mycelia. No sign of parasitism was noted in extensive areas where the two types of mycelia were closely intermixed.

Brefeld's statement that *Conidiobolus utriculosus* subsists parasitically on fruiting bodies of "Tremellinen" was not amplified by any mention of observed abnormal changes in the fructifications harboring the entomophthoraceous fungus. Couch made no mention of any abnormality affecting the *Septobasidium* material from which he obtained *C. brefeldianus*. White (1937) did not record any unusual condition in the apothecia of *Peziza domiciliana* Cooke which when fastened above an agar plate for ascospore discharge gave him abundant growth of *Delacroixia coronata*. In these several instances of adventitious occurrence of readily culturable entomophthoraceous fungi the fruiting bodies need not have been infected, but may merely have been newly contaminated with conidia cast upon them from neighboring mycelia of the phycomycetous forms concerned. Other objects within range of spore discharge, as, for example,

chunks of bark, fragments of wood, pieces of twigs, and lumps of leaf residues, could well be expected to become contaminated no less frequently than fruiting bodies of ascomycetes and basidiomycetes, but in the past have less often been superposed over nutrient solutions and sterile agar plates. Conidia adhering to them have had correspondingly less opportunity to discharge secondary conidia down upon an expanse of favorable substratum that was being kept under close observation by an alert investigator. Presumably neither newly discharged conidia nor actively sporulating mycelia are necessary in canopied plate cultures, since here the moisture in the soft agar used as an adhesive matrix encourages germination of resting spores.

A SLOW-GROWING LUSTROUS DISJUNCTIVE SPECIES WITH SMALL CONIDIA AND SMALL ZYGOSPORES

An unobtrusive species of *Conidiobolus* which in the small size of its conidia recalls *C. minor* was obtained from leaf mold collected on January 22, 1952, in woods near Fort Myer, Arlington, Va. Its isolation in pure culture was attended with some little difficulty, as its vegetative growth is slow in comparison with that of several congeneric species among which it was intermingled. When cultivated on moderately firm maizemeal agar at temperatures near 20° C., it extends its mycelium radially only about 2.5 mm in 24 hours. To the naked eye an individual young mycelium appears markedly lustrous throughout. Later as the mycelium expands the lustrous effect often diminishes in the older central region while remaining undimmed toward the sharply demarcated margin. When viewed under the microscope the hyphae in the marginal zone show a considerable degree of prallelism in their arrangement. For the most part they vary in width from 4 to 7μ (Fig. 1, A, B). Although the individual filament shows noticeable variations in width along its slightly crooked course, pronounced fluctuations in this dimension are not usual, and only rather little tapering is observable near the bluntly rounded tip. Branching at the margin of an extensive mycelium is often characterized by angular relationships usual in dichotomy (Fig. 1, B). Cross-walls are laid down fairly early, the most distal septum in a filament being often found 150 to 200μ from the tip. Vacuolization near a newly inserted cross-wall (Fig. 1, B) commonly leads to complete emptying of a short hyphal part, and as evacuated portions of hyphal membrane usually soon fade from sight many living segments appear disjointed from their fellows. Some disjointed segments later produce short diverticulate branches (Fig. 1, C) and thus acquire an irregular, somewhat lobulate outline.

Asexual reproduction takes place by development of a single conidium from the individual hyphal segment. A hyphal segment formed on the surface of the substratum pushes forth into the air and toward the main source of light an erect or ascending branch which on attaining a length frequently of 20 or 25μ (Fig. 1, D, E) swells out markedly at its tip. The terminal swelling receives all the protoplasmic contents of the hyphal segment, and is then delimited as a conidium through deposition of a convex basal wall. Hyphal segments formed in submerged positions first extend a branch or prolongation through the ambient to the surface. When the surface is reached the elongating filament grows erectly or ascendingly into the air, its course, after an abrupt (Fig. 1, F) or more gradual (Fig. 1, G, H) upward turn, being directed toward the main source of light. The aerial prolongation then develops into a conidiophore in the same way as an aerial branch from a procumbent hyphal segment. Once the globose conidium has been cut off it exerts strong pressure upon the basal septum protruding convexly upward, until the peripheral membrane ruptures circularly along the circumference of the partition. Immediately the basal wall splits into two layers, and concomitantly the distal layer is everted with such briskness through pressure of the conidial protoplast that the spore is thrown off forcibly, though the trajectory on a flat level surface may not exceed a few millimeters.

While in their small dimensions the conidia (Fig. 1, I, a–i), even without repetitional development, rather closely approach those shown in Brefeld's illustration of *Conidiobolus minor*, they seem less elongated than Brefeld's specimens, and their basal wall appears more abruptly protuberant. They are commonly filled throughout with coarsely granular protoplasm, except that the basal protuberance usually shows more nearly homogeneous texture. They do not nor-

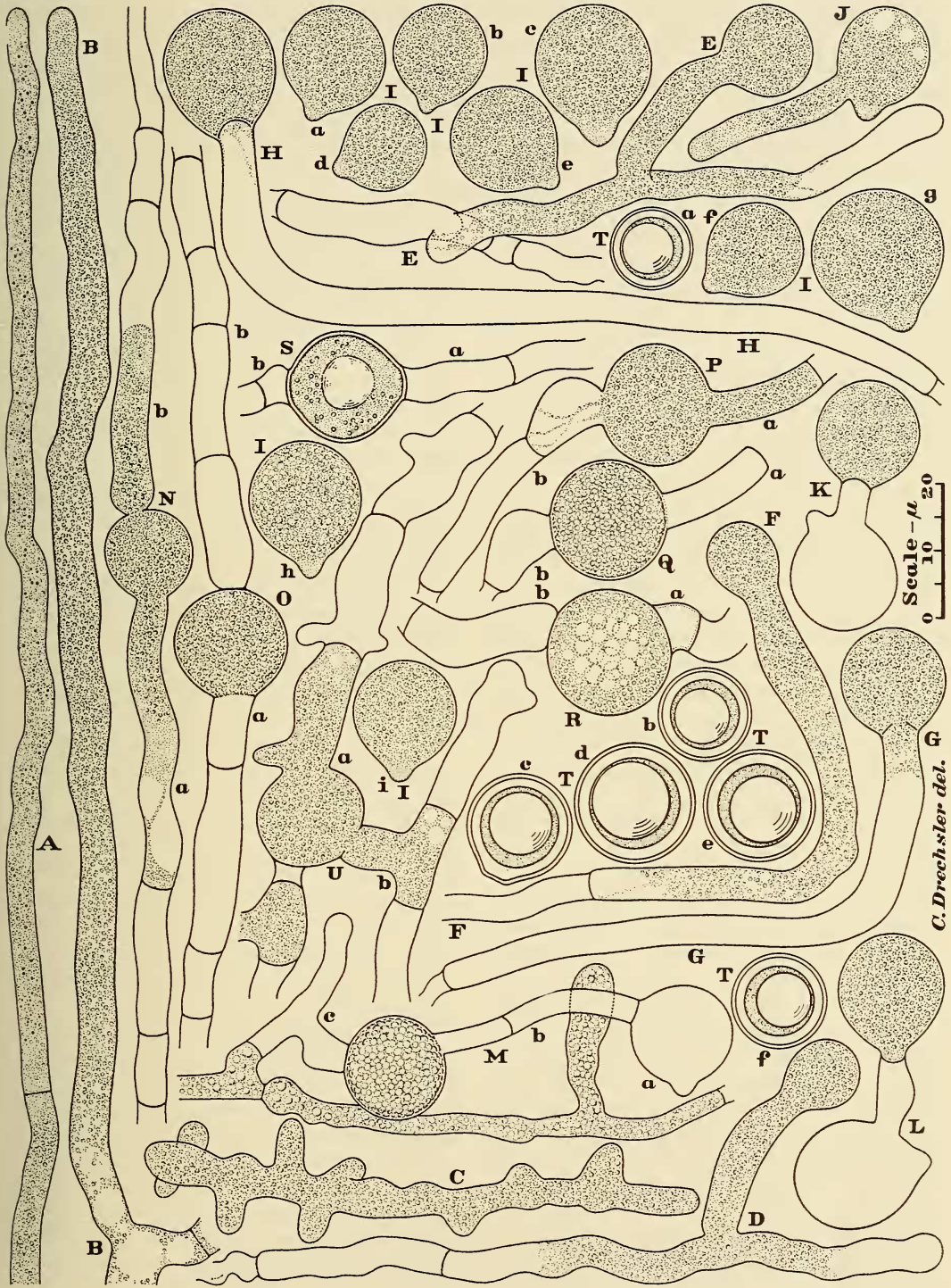

FIG. 1.—*Conidiobolus lamprauges*, sp. nov., as found developing in pure culture on Petri plates of maizemeal agar; all parts drawn at a uniform magnification with the aid of a camera lucida; × 1,000 throughout. Explanation of all parts given in text.

mally contain anything at all resembling the subspherical globules, about 3 to 5μ in diameter, shown in two conidia of *C. minor* depicted by Brefeld.

After falling on a moist substratum the discharged conidium often germinates by emission of a vegetative germ hypha (Fig. 1, J). If the substratum is already permeated with mycelium, repetitional development often ensues; the conidium putting forth a relatively short stout outgrowth on the tip of which it then gives rise to a secondary conidium (Fig. 1, K, L). The secondary conidium, like its parent, is normally delimited by a convexly arched basal partition (Fig. 1, K), and like its parent, again, is thrown off forcibly on circumscissile rupture of the peripheral membrane, abrupt splitting of the basal wall, and concomitant rapid eversion of the distal layer. In material mounted under a cover glass for microscopical examination all conidia, including those of secondary origin (Fig. 1, L), are usually not discharged after a normal manner, but commonly remain seated on the tip of the empty conidiophore, there gradually assuming their familiar proximally protuberant shape by gradually everting the entire basal septum.

Occasionally a conidium (Fig. 1, M, a) puts forth a germ tube (Fig. 1, M, b) that unites with a hyphal segment (Fig. 1, M, c) to form a zygospore. Much more often zygospores are formed through union of two hyphal segments that represent adjacent cells of the same mycelial filament. Onset of sexual reproductive development is first noticeable when one of the paired segments becomes locally swollen in the region near the crosswall separating it from its mate. The swelling increases steadily in size and soon appears as a globose enlargement. Apparently the adjoining portion of the other segment undergoes some widening at about the same time, but the increase in diameter here is usually less than 2μ, and only occasionally as much as 3μ. At a rather early stage the cross-wall separating the paired segments largely disappears, and granular protoplasm thereupon flows into the globose enlargement at both poles. Meanwhile the farther portions of both segments show increasing vacuolization, leading soon to progressive evacuation of contents. Successive stages in evacuation of the "female" segment—the segment (Fig. 1, N, a; O, a) within which the globose enlargement, or young zygospore, is formed—as well as of the "male" segment (Fig. 1, N, b; O, b) is frequently marked by deposition of a series of retaining walls. Transfer of protoplasm from the two segments is usually completed at nearly the same time, though in many instances the "female" segment (Fig. 1, P, a) appears somewhat slower than the "male" (Fig. 1, P, b) in contributing the last installment of its contents. As a rule the portions of membranous envelope successively evacuated soon collapse and vanish from sight, so that when the protoplasmic materials have migrated into the young zygospore, only relatively small membranous parts of the "female" (Fig. 1, Q, a; R, a) and "male" (Fig. 1, Q, b; R, b) segments remain visible. The portion of membranous envelope representing the "female" segment (fig. 1, S, a) shows no narrowing where it is attached, while that representing the "male" segment (Fig. 1, S, b) usually appears somewhat narrowed at its juncture with the zygospore, owing to the slight local enlargement of this segment at an early stage.

Transfer of protoplasm from the paired segments to the globose fusion cell is accomplished ordinarily in less than 2 hours. The subsequent changes in internal organization take place more slowly. By imperceptible stages the contents of the fusion cell change from a finely granular to a coarsely granular texture (Fig. 1, Q). Globules of increasing size appear near the center of the protoplast (fig. 1, R). These coalesce into a single reserve globule which at first is often of somewhat irregular shape (Fig. 1, S), but later, in the fully mature zygospore, has a sharply defined circular contour (Fig. 1, T, a–f). In the mature zygospore the thin wall originally present is found reinforced by a conspicuously thicker inner layer, and the living protoplasm forms a layer of nearly homogeneous consistency between the wall and the reserve globule.

The ripe zygospore here thus has much the same internal organization as the homologous spores of *Conidiobolus utriculosus* and *C. brefeldianus*. However the curious though specious resemblance that the sexual apparatus of *C. brefeldianus* bears to sexual reproductive apparatus of monosporous oomycetes is not evident in the present fungus. Even in the occasional instances where conjugation takes place between hyphal segments originating in separate hyphae (Fig. 1, U, a, b) fertilization of an oogonium by an antheridium is never closely simulated. Owing to early fusion of all paired hyphal segments any globose enlargement with conformation and

dimensions suggestive of an oogonium has very obviously received its contents in approximately equal measure from both segments.

A term (λαμπρανγης) meaning "lustrous" may serve conveniently as specific epithet of the fungus in bringing to mind the macroscopic appearance of its mycelium.

Conidiobolus lamprauges, sp. nov. Mycelium lente crescens (circa 2.5 mm in die), incoloratum, nitidum, aliquid ramosum, mox septatum, in hyphis 3–8μ (plerumque 4–7μ) latis constans; cellulis mycelii 35–200μ longis, saepius aliquid flexuosis, quandoque plus minusve disjunctis, interdum pluribus ramulis brevibus praeditis; hyphis conidiophoris simplicibus, erectis vel ascendentibus, in aere 25–100μ (vulgo 25–50μ) ad lucem protendentibus, interdum 5–15μ subter apicem parum inflatis, ibi 4–8μ latis, in apice unicum conidium ferentibus; conidiis se violenter abjicentibus, incoloratis, globosis, sed deorsum papilla rotundoconica vel hemisphaerica (1.5–4μ alta, 2.5–7μ lata) praeditis, 15–22μ (ex toto) longis, 12.5–20μ crassis, protoplasmatis dense granulosi repletis; zygosporis interdum e copulatione inter cellulam mycelii et tubum germinationis interdum e copulatione cellularum aliae atque aliae hyphae ortis, sed saepissime e copulatione cellularum duarum contiguarum ejusdem hyphae oriundis, hyalinis, globosis, plerumque 12–18μ crassis, in maturitate guttula nitida 7.5–11.5μ crassa et muro 1.3–2.2μ crasso praeditis.

Habitat in foliis quercorum putrescentibus in Arlington, Virginia.

Mycelium colorless, lustrous, at 20° C. growing radially about 2.5 mm in a day, moderately branched; assimilative hyphae somewhat flexuous, 3 to 8μ (mostly 4 to 7μ) wide, soon becoming divided by cross-walls at intervals of 35 to 200μ; the resulting hyphal segments sometimes remaining contiguous and at other times becoming disjointed, frequently after disjunction putting forth several short diverticulate or lobate branches. Conidiophores arising singly from individual hyphal segments, simple, colorless, projecting 25 to 100μ (commonly 25 to 50μ) erectly or ascendingly into the air, the aerial part always oriented toward the main source of light, often slightly widened and having a diameter of 4 to 8μ some little distance (mostly 5 to 15μ) below its tip whereon is borne a single conidium. Conidia filled with densely granular protoplasm, through sudden eversion of the upcurved basal membrane forcibly thrown off, colorless, globose, measuring 15 to 22μ in total length and 12.5 to 20μ in greatest width, the everted basal membrane forming a hemispherical or rounded-conical papilla 1.5 to 4μ high and 2.5 to 7μ wide at its origin. Conjugation sometimes taking place between a germ hypha and a hyphal segment, sometimes between 2 hyphal segments originating in separate mycelial filaments, but most often between 2 adjacent segments in the same mycelial filament; the fusion cell always initiated wholly within one of the gametangia, though in immediate proximity to the other gametangium; zygospore at maturity hyaline, globose, usually 12 to 18μ in diameter, containing an eccentrically placed reserve globule 7.5 to 11.5μ in diameter, provided with a wall commonly 1.3 to 2.2μ thick.

Occurring in decaying oak (*Quercus* spp.) leaves in woods in Arlington, Va.

A SPECIES WITH INCONSPICUOUS DISJUNCTIVE MYCELIUM AND PREDOMINANTLY DICLINOUS CONJUGATION

A species of *Conidiobolus* noticeably more robust than *C. lamprauges* was obtained from leaf mold kindly collected by A. W. Rakosy in Carroll County, N. H., late in September 1951. In maizemeal-agar plate cultures kept at temperatures near 20° C. it grows radially about 5 mm in 24 hours. Its submerged mycelium is inconspicuous, frequently being only indistinctly visible to the naked eye except at the sharply demarcated advancing margin, though it never vanishes from macroscopic sight as completely as the mycelium of two species of *Basidiobolus* that are widely distributed in leaf mold (Drechsler, 1952a). Viewed under the microscope an expanding mycelium of the fungus displays at its periphery terminal portions of many elongating hyphae mostly 6 to 8μ in width (Fig. 2, A). Very little tapering is observable below the bluntly rounded end. Formation of cross-walls ensues after about an hour, with the result that in many hyphae the most distal septum is found approximately 200μ from the tip. The segments delimited successively in the individual filaments vary moderately in length. Many are a little longer (fig. 2, J, a) or a little shorter (Fig. 2, J, b) than 100μ. As in *C. lamprauges* hyphal segments formed adjacent to one another may remain contiguous or may become disjointed through withdrawal of contents from one side of the separating cross-wall. After being delimited some segments will

widen perceptibly, then occasionally attaining a diameter in excess of 10μ (Fig. 2, B, a). In addition such stout segments not infrequently will put forth branches only 3 or 4μ wide (Fig. 2, B, b, c) and will thereby in small compass display opposite extremes in thickness of filamentous parts.

Asexual reproduction takes place abundantly in maizemeal agar cultures of the fungus. An individual hyphal segment that is immersed under the substratum extends a branch or prolongation which on reaching the surface soon turns upward and after widening rather markedly (Fig. 2, C, a) forms a globose swelling at its tip (Fig. 2, C, b). A hyphal segment that has originated in a procumbent hypha often puts forth a branch erectly or ascendingly into the air(Fig. 2, D, a). This aerial branch, much like the aerial termination of a branch from a submerged segment, widens out markedly and then forms at its summit a globose swelling (Fig. 2, D, b) into which are soon received the entire protoplasmic contents of the reproductive unit. Thereupon the arched septum that was being formed progressively at the base of the globose part—its formation proceeding from the periphery inward—during the later stages in the upward movement of protoplasm, is completed through deposition of wall material in the keystone region. A subspherical conidium is thus delimited, and soon afterwards is thrown off violently on sudden eversion of the distal layer of the arched partition. Since the aerial conidiophores, as in related species, are in conspicuous degree positively phototropic, the direction of discharge is consistently toward the main source of light.

The conidia of the New Hampshire fungus (Fig. 2, E, a–j) are in general larger than those of *Conidiobolus lamprauges*. Often the basal membrane here (Fig. 2, E, a–c) would seem to protrude less abruptly from the globose outline of the spore than in *C. lamprauges*, but often, too, the everted wall protrudes hardly less markedly (Fig. 2, E, d–h) than in the latter species. Usually the conidia of *C. lamprauges* seem filled throughout with coarsely granular protoplasm, whereas those of the New Hampshire fungus show commonly a relatively clear peripheral layer that surrounds a large mass of conglutinated lumps. The lumps, varying in width from 1.5 to 3.5μ, have an irregularly globose shape and thus somewhat resemble small oil globules, but unlike oil globules are little given to coalescence. A conidium may germinate vegetatively by putting forth a germ hypha (Fig. 2, F) or it may extend a conidiophore of variable length and produce a secondary conidium (Fig. 2, G, H).

Sexual reproduction is accomplished by conjugation so simple that the general appearance given differs little from that of chlamydospore development. In some instances two adjoining segments of the same hypha (Fig. 2, I, a, b) serve as gametangia, the fusion cell arising as a globose swelling situated wholly within one segment but lying immediately adjacent to the other segment. The separating cross-wall disappears almost entirely at an early stage, so that the incipient enlargement soon receives protoplasmic materials from both directions. As a rule the "male" segment (Fig. 2, I, b) may be distinguished from the "female" (Fig. 2, I, a) by its narrower attachment to the young fusion cell. Conjugation between adjacent segments apparently occurs less frequently in the present species than scalariform conjugation between segments of different hyphae (Fig. 2, J, a, b). Since in diclinous reproductive apparatus, too, the fusion cell is initiated at the place of hyphal union and wholly within one of the two gametangia (Fig. 2, K) the zygote commonly develops in a position partly or wholly within the hyphal connection. At the place of union the apposed portions of outer membranes dissolve almost completely, so that here, just as in monoclinous apparatus, protoplasm flows into the young fusion cell from the "male" segment (Fig. 2, J, b) about as freely as from the "female" segment (Fig. 2, J, a). Indeed, the "male" segment (Fig. 2, J, b) will often have contributed all its contents when its mate (Fig. 2, J, a) still retains a considerable quantity of protoplasm. Soon after portions of conjugating hyphal segments have been evacuated the empty tubular membrane, together with the septa contained in it, collapses and disappears from view. Thus, only an hour after movement of protoplasm began in the unit of sexual apparatus shown in Fig. 2, J, less than a third of the original membranous envelope of the "male" segment (Fig. 2, K, b) and scarcely half of the original envelope of the "female" segment (Fig. 2, K, a) remained visible. Forty minutes later all membranous parts of the "male" gametangium had vanished, and only two short membranous spurs (Fig. 2, L, a), both left by the "female" gametangium, could be seen attached to the developing zygospore, which now had not only laid down its definitive delimiting walls but had begun internal reorganization by elabo-

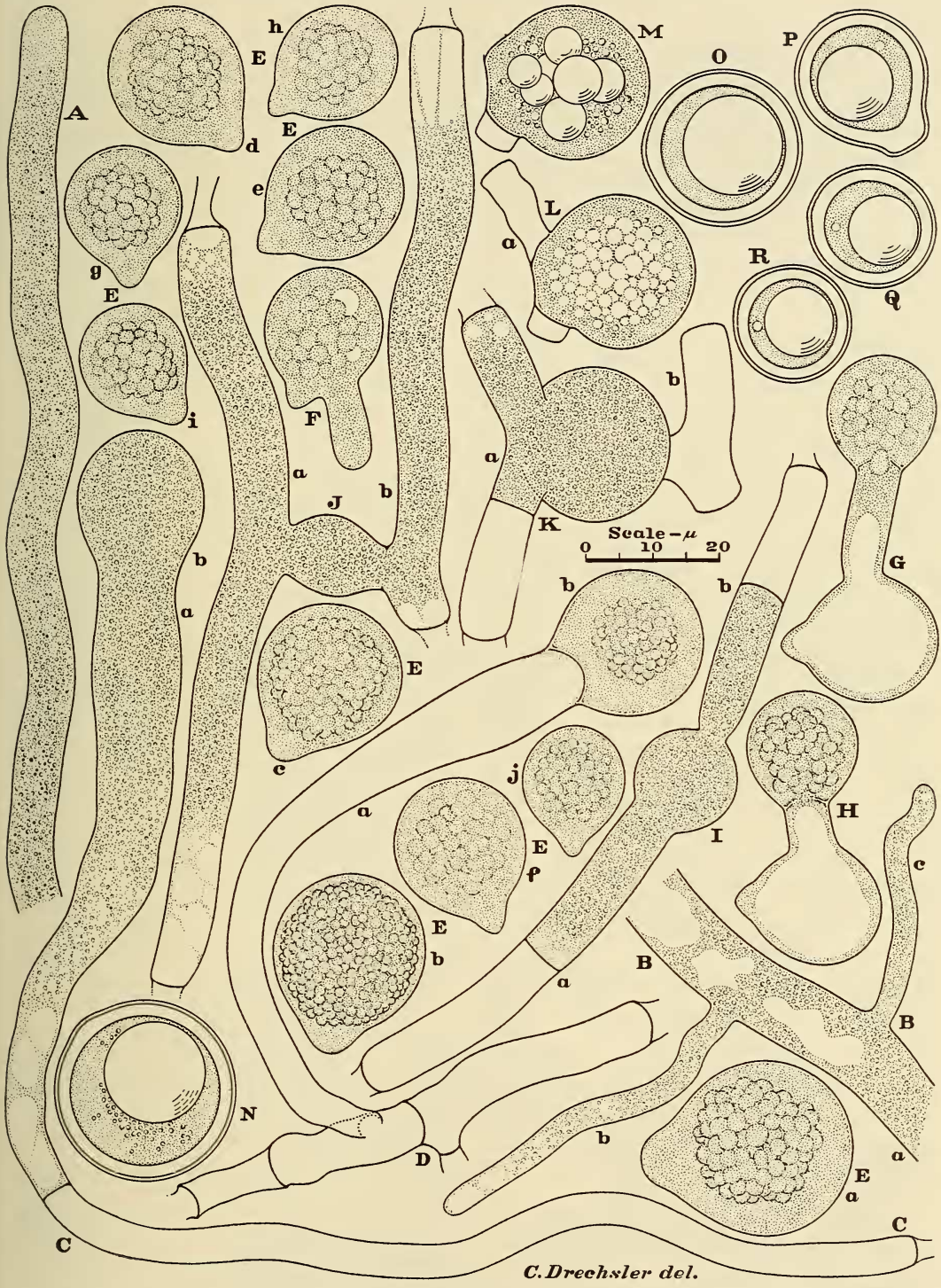

FIG. 2.—*Conidiobolus thromboides*, sp. nov., as found developing in pure culture on Petri plates of maizemeal agar; all parts drawn at a uniform magnification with the aid of a camera lucida; × 1000 throughout. Explanation of all parts given in text.

rating many oily globules in its central region. During the ensuing 30 minutes the longer of the two empty spurs vanished from sight, so that in little more than two hours after conjugation could be clearly ascertained the united hyphal segments were converted into a globose zygote (Fig. 2, M) with only a short empty cylindrical stub to indicate its origin from filamentous parts. The numerous small globules in the central region of the zygote had meanwhile coalesced to form seven or eight globules of considerably larger size.

Through continued coalescence of multiple globules a single large reserve globule is eventually formed. This body, much as in *Conidiobolus lamprauges*, lies well toward one side within the ripe zygospore (Fig. 2, N–R), a portion of its periphery approaching very close to the zygospore wall. Accordingly at maturity the protoplasm, which shows relatively few granules scattered in a limpid matrix of nearly homogeneous appearance, is disposed in a parietal layer pronouncedly thicker on one side than on the other. The thin envelope earlier surrounding the fusion cell is reinforced in the ripe zygospore by a much thicker inner layer presumably interpretable as the zygospore wall proper. Variability with respect to size is more moderate among zygospores of the New Hampshire fungus than might be inferred from the five individuals figured herein (Fig. 2, N–R), for one (Fig. 2, N) of the five—a specimen fully 27μ in diameter—was selected more especially to illustrate approximately maximum dimensions, while two others (Fig. 2, Q, R), each about 18μ in diameter, were selected to illustrate approximately minimum dimensions. Only two (Fig. 2, O, P) of the five individuals, with diameters of 23.5μ and 20μ, respectively, are of dimensions frequent in the species.

A term (*θρομβοειδης*) meaning "full of of clots or grains" may serve helpfully as specific epithet in recalling the conglutinated lumpy texture of conidial contents wherein the fungus differs markedly from the generally smaller *Conidiobolus lamprauges*.

Conidiobolus thromboides sp. nov. Mycelium circa 5 mm in die crescens, incoloratum, saepius parum conspicuum, aliquid ramosum, mox septatum, in hyphis 3–10.5μ (saepe 6–8μ) latis constans; cellulis assumentibus 50–200μ (saepe circa 100μ) longis, vulgo aliquid flexuosis, aliquando plus minusve disjunctis, interdum paucis angustis ramulis praeditis; hyphis conidiophoris simplicibus, erectis vel ascendentibus, in aere vulgo 35–150μ ad lucem protendentibus, sursum inflatis, ibi saepe 10–15μ latis, in apice unum conidium gignentibus; conidiis se violenter abjicentibus, incoloratis, globosis sed basi papilla rotundoconica vel hemisphaerica (2.5–6μ alta, 4–10μ lata) praeditis, plerumque ex toto 24–32μ longis, 19–26.5μ latis, in magna parte praecipue in medio glebarum protoplasmatis conglutinatarum repletis; zygosporis interdum e copulatione cellularum aliae atque aliae hyphae interdum e copulatione cellularum duarum contiguarum ejusdem hyphae oriundis, hyalinis, globosis, 17.5–27μ (plerumque 19.5–23.5μ) crassis, in maturitate guttula nitida 10–15μ crassa et muro magnam partem 2–2.5μ crasso praeditis.

Habitat in humo silvatica in New Hampshire.

Mycelium colorless, often rather inconspicuous, moderately branched, at temperatures near 20° C. growing radially about 5 mm in a day; assimilative hyphae somewhat flexuous, 3 to 10.5μ (mostly 6 to 8μ) wide, soon becoming divided by cross-walls at intervals of 50 to 200μ; the hyphal segments sometimes remaining contiguous but at other times becoming disjointed, and in some instances putting forth one or more narrow branches. Conidiophores arising singly from individual hyphal segments, simple, colorless, projecting 35 to 150μ (often about 100μ) erectly or ascendingly into the air, the aerial part oriented toward the main source of light, distally inflated, often measuring 10 to 15μ in greatest width, bearing a single terminal conidium. Conidia forcibly thrown off through sudden eversion of the arched basal membrane, colorless, usually in large part filled with somewhat conglutinated protoplasmic lumps, globose, often measuring 24 to 32μ in total length and 19 to 26.5μ in greatest width, the everted basal membrane forming a hemispherical or rounded-conical papilla 2.5 to 6μ high and 4 to 10μ wide at its origin. Conjugation most usually taking place between two hyphal segments originating in separate mycelial filaments but sometimes taking place between two adjacent segments in the same filament; the fusion cell always initiated wholly within one segment and in immediate proximity to the other; zygospore at maturity hyaline, globose, 17.5 to 27μ (mostly 19.5 to 23.5μ) in diameter, containing a very eccentrically placed reserve globule 10 to 15μ in diameter, and provided with a wall for the most part 2 to 2.5μ thick.

Occurring in leaf mold in Carroll County, N. H.

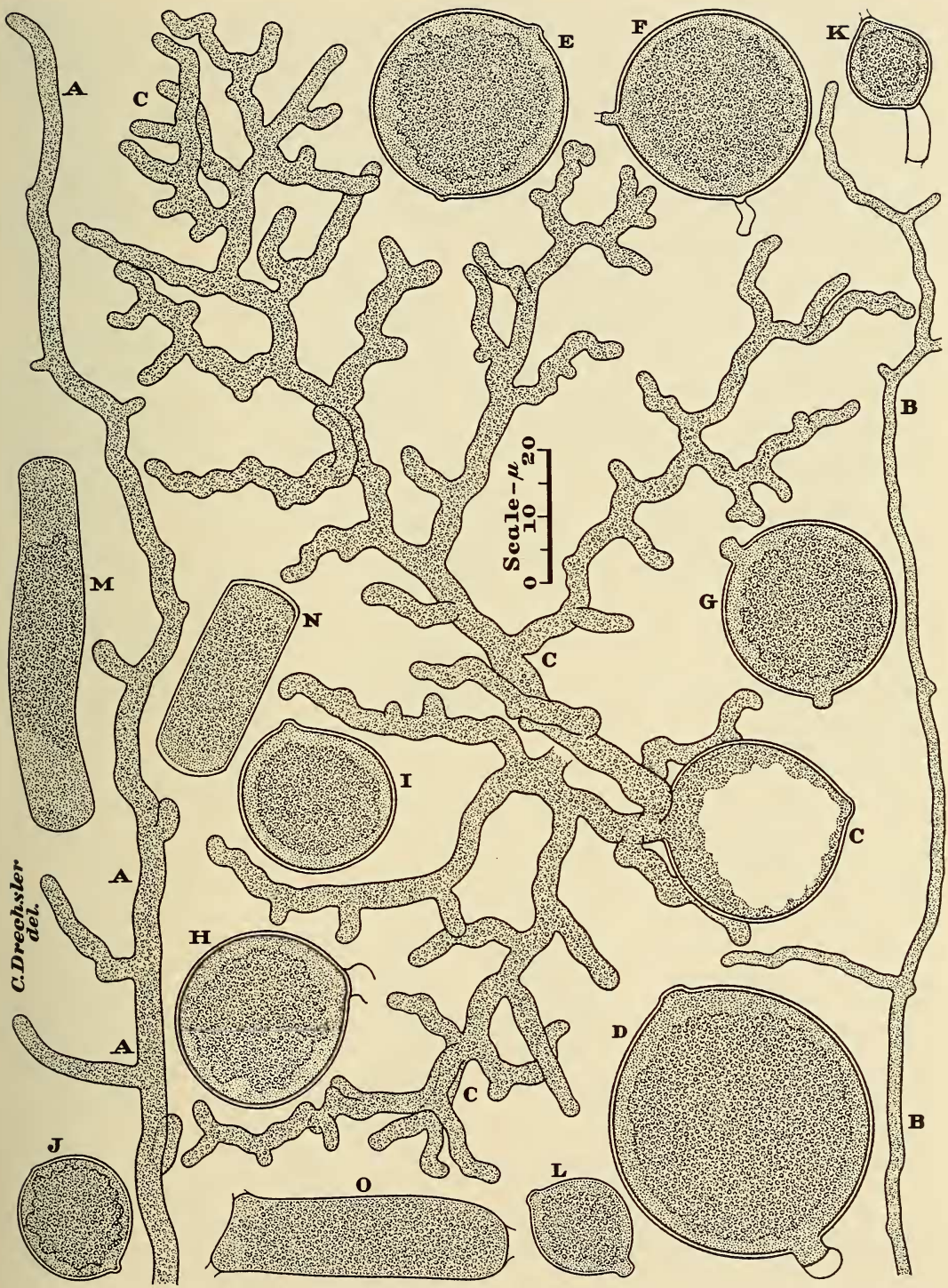

FIG. 3.—*Conidiobolus adiaeretus*, sp. nov., as found developing in pure culture on Petri plates of maizemeal agar; all parts drawn at a uniform magnification with the aid of a camera lucida; × 1000 throughout. Explanation of all parts given in text.

A SPECIES WITH ROBUST CONIDIOPHORES ARISING FROM RICHLY BRANCHED DELICATE MYCELIUM

An entomophthoraceous fungus especially distinctive in its vegetative stage was first found developing in agar plate cultures prepared with leaf mold collected near Farmer, N. C., in December 1951. Subsequently it was obtained also from leaf mold gathered in eastern central New Hampshire late in September 1951; from leaf mold gathered in oak woods along Lubbers Run in Arlington, Va., on February 28, 1952; from leaf mold collected near Criglersville, Va., on March 23, 1952; and from various kinds of plant detritus taken up in several places near Beltsville, Md., at different times during January and February 1952. On maizemeal agar of moderate firmness it grows slowly, producing a lustrous mycelium somewhat more nearly opaque and correspondingly more conspicuous than the mycelium of *Conidiobolus lamprauges*. Before an individual mycelium has spread extensively it produces conidiophores from which conidia are thrown for distances of several millimeters toward the main source of light. Falling on a moist substratum many of these conidia give rise collectively to scattered subsidiary mycelia which soon occupy the area completely thereby barring further growth in that region by the parent mycelium. As the same sequence of events is repeated another array of mycelia come into being a little farther onward, which in their turn form a barrier against those to their rear. The fungus thus spreads over an expanse of substratum by establishing numerous demarcated mycelia that in large part remain discernible as individuals and therefore in the end often appear collectively as a patchwork of lustrous areas. Ordinarily no similar patchy or dappled effect is noticeable in related fungi, for while these likewise habitually colonize adjacent areas, their outlying mycelia—often from the first too transparent to stand out individually in clear relief—become merged indistinguishably when they coalesce.

Under a microscope an extensive unobstructed mycelium of the present fungus shows along its growing margin numerous hyphae that measure mostly 3.5 to 4μ in width, though in the distal portion they taper gradually to an apical width of approximately 3μ (Fig. 3, A). Here and there in older cultures narrower hyphae are found which over considerable stretches may not exceed 2μ in width and, indeed, may in some portions measure as little as 1.8μ in this dimension (Fig. 3, B). The greatest width sustained for some distance in the stouter filaments would seem approximately 4.5μ (Fig. 4, A). Only rather moderate development of lateral branches occurs at the margin of an extensive mycelium (Fig. 3, A). Abundant branching is, however, usual in the earlier development of a mycelium from a germinating conidium. The ramified procumbent outgrowths shown in Fig, 3, C, represent only about one-twentieth of the entire three-dimensional hyphal system formed within a radius of 150μ from an individual spore.

In older portions of an extensive mycelium many of the lateral branches (Fig. 4, A, r; B, r) are empty of protoplasmic contents and accordingly are found delimited basally from the parent hypha by a retaining wall. A much smaller number continue growth distally to give rise to conidiophores (Fig. 4, A, a; B, a; C, a). As the conidiophores here are often 20μ or more in greatest width they offer a pronounced dimensional contrast with the mycelial filaments. The prolonged transfer of granular materials into the growing terminal conidium (Fig. 4, B, b) is not regularly accompanied, as in related species, by evacuation of a particular hyphal segment, or of any adjoining portion of axial hypha. When eventually the conidiophore is delimited by a basal septum (Fig. 4, C, a) the axial hypha and the connecting branch are often still filled with granular protoplasm. An arched septum is progressively laid down at the base of the conidium (Fig. 4, C, b) during the later stages in the upward movement of living contents. Soon after the septum has been completed, it is suddenly split into two layers. The distal layer at the same time is briskly everted, with the result that the conidium is thrown off forcibly. The distances spores are propelled here seem appreciably less than in *Delacroixia coronata* and *Conidiobolus brefeldianus*. Feebler propulsion might readily be expected since in my fungus the basal septum is arched less prominently, and therefore in being everted delivers a shorter and presumably less powerful stroke.

The largest of the primary conidia (Fig. 4, D) produced by the fungus measure approximately 46μ in total length and 45μ in width. Well developed primary conidia commonly vary between 30 and 40μ in both dimensions (Fig. 4, E, F). Individuals less than 25μ (Fig. 4, G–M) would mostly seem to represent products of repetitional development. Such development

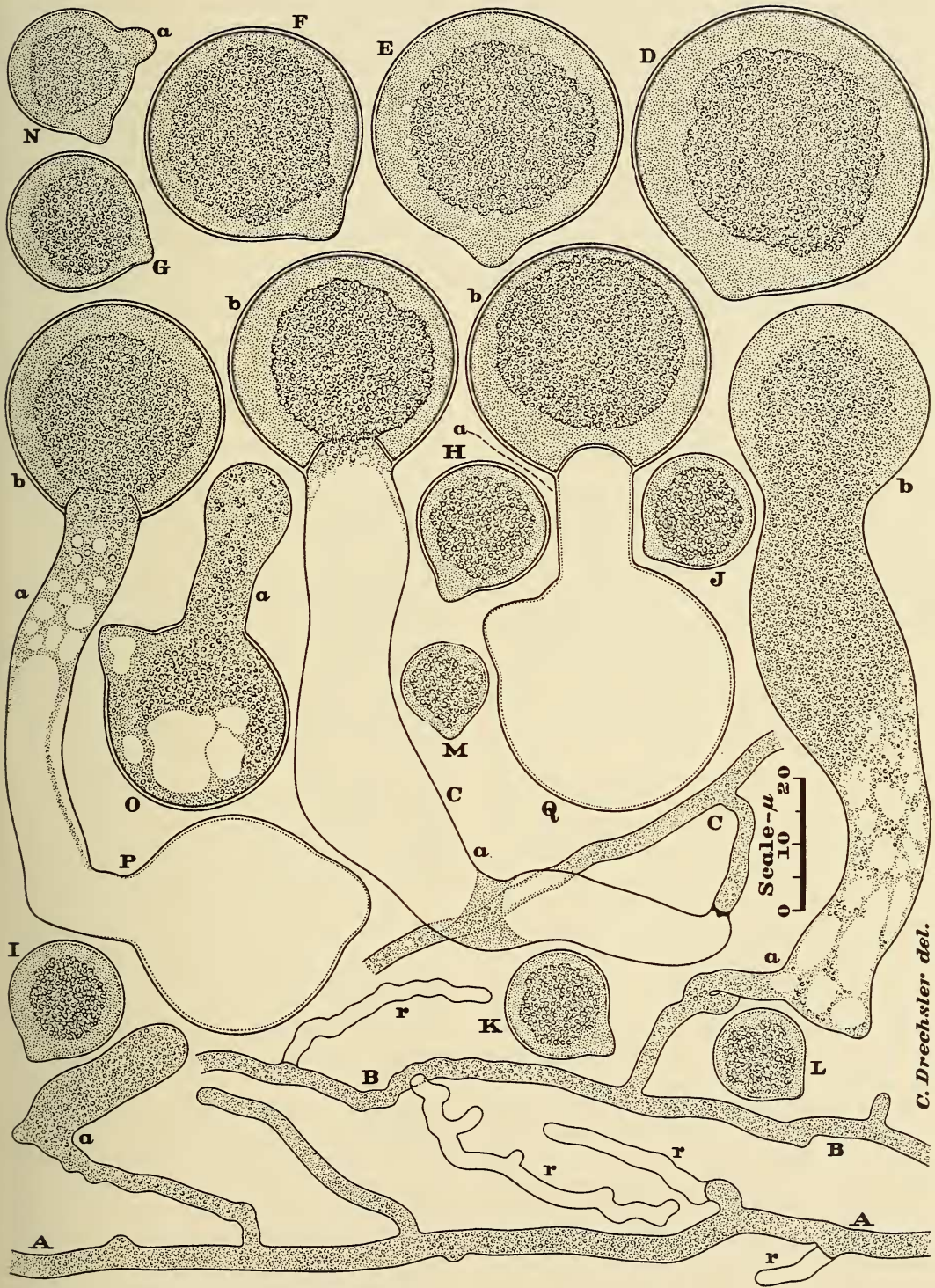

FIG. 4.—*Conidiobolus adiaeretus*, sp. nov., as found developing in pure culture on Petri plates of maizemeal agar; all parts drawn at a uniform magnification with the aid of a camera lucida; × 1000 throughout. Explanation of all parts given in text.

takes place very freely in the present species. A protuberance is bourgeoned forth (Fig. 4, N, a) which after some elongation (Fig. 4, O–Q: a) swells distally to form a globose secondary conidium (Fig. 4, P, b; Q, b) that is thrown off forcibly in the same way as was its parent. As the reduction in size incurred in one repetitional generation is not especially pronounced in instances where the conidiophorous outgrowth is of moderate length, many secondary conidia (Fig. 4, P, b; Q, b) measure 30 to 40μ in diameter and thus after discharge are not distinguishable from well developed primary conidia. With respect to their internal organization the conidia, both large and small somewhat resemble those of *Conidiobolus thromboides* in having a rather dense conglutinated central mass of protoplasm surrounded by a hyaline parietal layer. However the central mass here is less coarse in texture, its constituent particles being granules rather than lumps. When conidia are mounted in moist agar under a cover glass and subjected to microscopical examination in strong light the conglutinated mass soon contracts noticeably and the clear parietal layer becomes interspersed with vacuoles of increasing number and size.

At temperatures near 20° C. aerial conidia are the only reproductive bodies formed by the fungus. On being stored at temperatures near 7° C. tubes of maizemeal agar well permeated with mycelium will permit copious formation of chlamydospores mainly under the surface of the culture medium. Since these chlamydospores, usually of globose or prolate ellipsoidal shape, very often show two truncated protuberances in opposite positions (Fig. 3, D–G), they appear largely of intercalary origin. Specimens showing only one protuberance suggestive of hyphal attachment (Fig. 3, H, I) seem of terminal origin. Most chlamydospores, like most conidia, vary in diameter from 25 to 40μ (Fig. 3, D–H). Small individuals (Fig. 3, I–L), corresponding in their dimensions to conidia derived through successive repetitional development, are usually found only in meager quantity. Somewhat indurated cylindrical cells (Fig. 3, M–O), often about three times as wide as unmodified assimilative hyphae, are perhaps to be regarded as imperfectly differentiated chlamydospores. They often show conglutinated granules in the middle region and clear protoplasm at both ends (Fig. 3, M, N). In well differentiated globose chlamydospores, much as in conidia, a relatively large conglutinated granular mass is surrounded by a parietal layer of more nearly transparent protoplasm.

The fungus is referred to *Conidiobolus* since it grows well on ordinary culture media and in its asexual reproduction does not differ very widely from *C. utriculosus*, the type species of that genus. Its mycelium differs conspicuously from that of *C. utriculosus*, *C. brefeldianus*, *C. lamprauges*, and *C. thromboides* not only in the slenderness of the component hyphae but also in their frequently copious branching. Although the fungus forms numerous septa that serve as retaining walls in closing off evacuated lateral branches from the living axial hyphae, early deposition of cross-walls to separate adjacent living segments—a very usual feature in the vegetative growth of other readily cultivable Entomophthoraceae—is not characteristic of its mycelial development. In agar plate cultures it shows no disjunction of living hyphal segments. A term (αδιαιρετος) meaning "undivided" is therefore deemed a suitable specific epithet.

Conidiobolus adiaeretus, sp. nov. Mycelium lente (circa 2 mm in die) crescens, nitidum, conspicuum; hyphis assumentibus, incoloratis, vulgo 1.8–4.5μ latis; interdum mediocriter interdum copiose ramosis, ramulis brevibus saepe mox inanitis denique ab hyphis viventibus longis septo finitis; hyphis conidiophoris incoloratis, simplicibus, erectis vel ascendentibus, in aere vulgo 50–100μ (rarius 100–250μ) ad lucem protendentibus, rectis vel curvatis, vulgo speciose inflatis, 8–25μ (saepius 15–23μ) latis, in apice unum conidium gignentibus; conidiis se violenter adjicentibus, incoloratis, globosis vel applanato-ellipsoideis sed basi papilla rotunda (2–6μ alta, 5–17μ lata) praeditis, plerumque ex toto 15–46μ longis, 13–45μ latis, in parte parietem juxta protoplasmatis hyalini repletis in parte media granulis conglutinosis farctis; chlamydosporis plerumque intra materiam permeatam oriundis, incoloratis, plerumque intercalaribus, interdum terminalibus, vulgo globosis vel elongato-ellipsoideis, 15–45μ longis, 3–40μ latis, in parte parietem juxta protoplasmate hyalino in parte media granulis conglutinosis instructis.

Habitat in foliis arborum (praecipue quercorum) putrescentibus prope Farmer, N. C., et prope Beltsville, Md., et prope Criglersville, Va., et in Arlington, Va., et in New Hampshire, etiam in aliis materiis plantarum putrescentibus prope Beltsville, Maryland.

Mycelium growing slowly (about 2 mm in 24 hours at 20° C.), lustrous, conspicuous; assimilative hyphae colorless, mostly 1.8 to 4.5μ wide, sometimes moderately and sometimes abundantly branched, the shorter branches often emptied early of their protoplasm and then delimited basally by a retaining wall; conidiophores colorless, simple, straight or curved, projecting 50 to 200μ (or more) erectly or ascendingly into the air, the aerial part oriented toward the main source of light, often pronouncedly inflated, 8 to 25μ (commonly 15 to 23μ) in greatest width, bearing a single conidium at the tip; conidia forcibly thrown off through sudden eversion of the arched basal membrane, colorless, containing a parietal layer of hyaline protoplasm which surrounds a large irregular mass of conglutinated granules, subspherical or sometimes oblate ellipsoidal in general shape, measuring 15 to 46μ in total length and 13 to 45μ in width, their everted basal membrane forming a rounded papilla 2 to 6μ high and 5 to 17μ wide; chlamydospores formed mainly within the substratum, borne intercalarily or terminally, mostly globose or ellipsoidal, 15 to 45μ long and 13 to 40μ wide, colorless, containing a large central mass of conglutinated granules that is surrounded by a parietal layer of hyaline protoplasm.

Occurring in decaying leaves of trees (especially of *Quercus* spp.) in woods near Farmer, N. C.; near Beltsville, Md.; near Criglersville, Va.; in Arlington, Va.; in Carroll County, N. H.; and also in other decaying plant materials near Beltsville, Md.

REFERENCES

BREFELD, O. Conidiobolus utriculosus *und* minor. Unters. Gesammtg. Mykologie **6:** 35–72, 75–78, pl. 3–5. 1884.

COUCH, J. N. *A new* Conidiobolus *with sexual reproduction.* Amer. Journ. Bot. **26:** 119–130. 1939.

DRECHSLER, C. *Widespread distribution of* Delacroixia coronata *and other saprophytic Entomophthoraceae in plant detritus.* Science **115:** 575–576. 1952.

———. *Two species of* Basidiobolus *widely distributed in leaf mold.* (Abstract) Phytopathology **42:** 341. 1952a.

GILBERT, E. M. *A peculiar entomophthorous fungus.* Trans. Amer. Micr. Soc. **38:** 263–269, pl. 27, 28. 1919.

WHITE, W. L. *Note on* Conidiobolus. Mycologia **29:** 148–149. 1937.

BOTANY.—*The species of* Pittosporum *in Formosa.* HUI-LIN LI, Morris Arboretum, University of Pennsylvania.

There are five species of the genus *Pittosporum* on the island of Formosa. Two of them are more or less widespread in Formosa and extend also widely on the mainland of China. One is confined to the southern part of the island, and another is found only on the small island of Botel Tobago. These two southern species described as endemic to Formos are actually found to be only the northernmost populations of two widely distributed Philippine species. A fifth species is endemic to Formosa at high altitudes only.

Recently a treatise on the *Pittosporum* species of eastern Asia was published by M. Gowda (*The genus* Pittosporum *in the Sino-Indian Region.* Journ. Arnold Arb. **32:** 263–343. 1951). Six species[1] from Formosa are accounted for, his findings being very much at variance with those of the present writer. Gowda considers the Formosan plant known as *P. makinoi* to be distinct, but in the present study it is treated as conspecific with the widely distributed *P. tobira.* For *P. illicioides* treated as a single species here, two separate species under different names are recognized by Gowda, one described as new. Gowda did not recognize the identity and relationship with Philippines species of the two southern species of Formosa. As *Pittosporum* is primarily a southern genus, the nature of some of the southernmost species in Formosa, on the Chinese mainland, and in India cannot be properly elucidated without consulting related species of the southern islands of Asia.

Selected specimens are cited from the U. S. National Herbarium, Smithsonian Institution, indicated as (US), and the herbarium of the National Taiwan University, Formosa, indicated as (NTU).

[1] On page 282, Gowda mentions that there are five species known from Formosa. He inadvertently left out his own new species, *P. sahnianum*, which he credited to Formosa on the basis of *Wilson 11066*.